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TCE:
Reactionary Regulatory Representation *versus* Realistic Reckoning of Relevant Exposures Rules Out Reasonable Clean Up Standards

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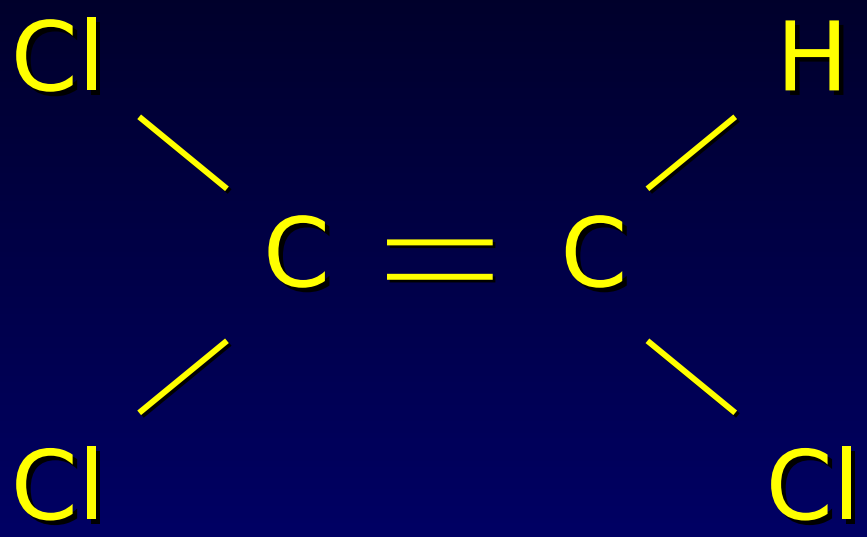
Trichloroethylene

(Trichloroethene; TCE; TRI)



- **Colorless DNAPL**
- **Aqueous solubility: ~1100 ppm (1.1 g/L)**
- **Odor threshold in water: ~ 28 ppm**
- **Vapors mildly addictive**

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Historical Uses of Trichloroethylene

- Medical applications
  - Anesthetic agent
  - Analgesic for trigeminal neuralgia
  - Disinfectant
- Extraction solvent for foods
- Solvent in manufacture of cosmetics
- Dry cleaning solvent

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Current Uses of Trichloroethylene

- Metal degreasing agent
- Manufacturing solvent
  - Pesticides
  - Varnishes, lacquers, paints
  - Dyes
- Component of
  - Adhesives
  - Spot removers
  - Rug cleaners
  - Disinfectants

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Why should TCE be evaluated?

- Occupational exposures
- Environmental contaminant
  - Widespread
  - Persistent (in groundwater environments)
  - Mobile

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What major diseases are alleged?

- Noncancer
  - Cardiovascular birth defects
  - Small for gestational age
- Cancer
  - Liver
  - Kidney

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Metabolism of TCE

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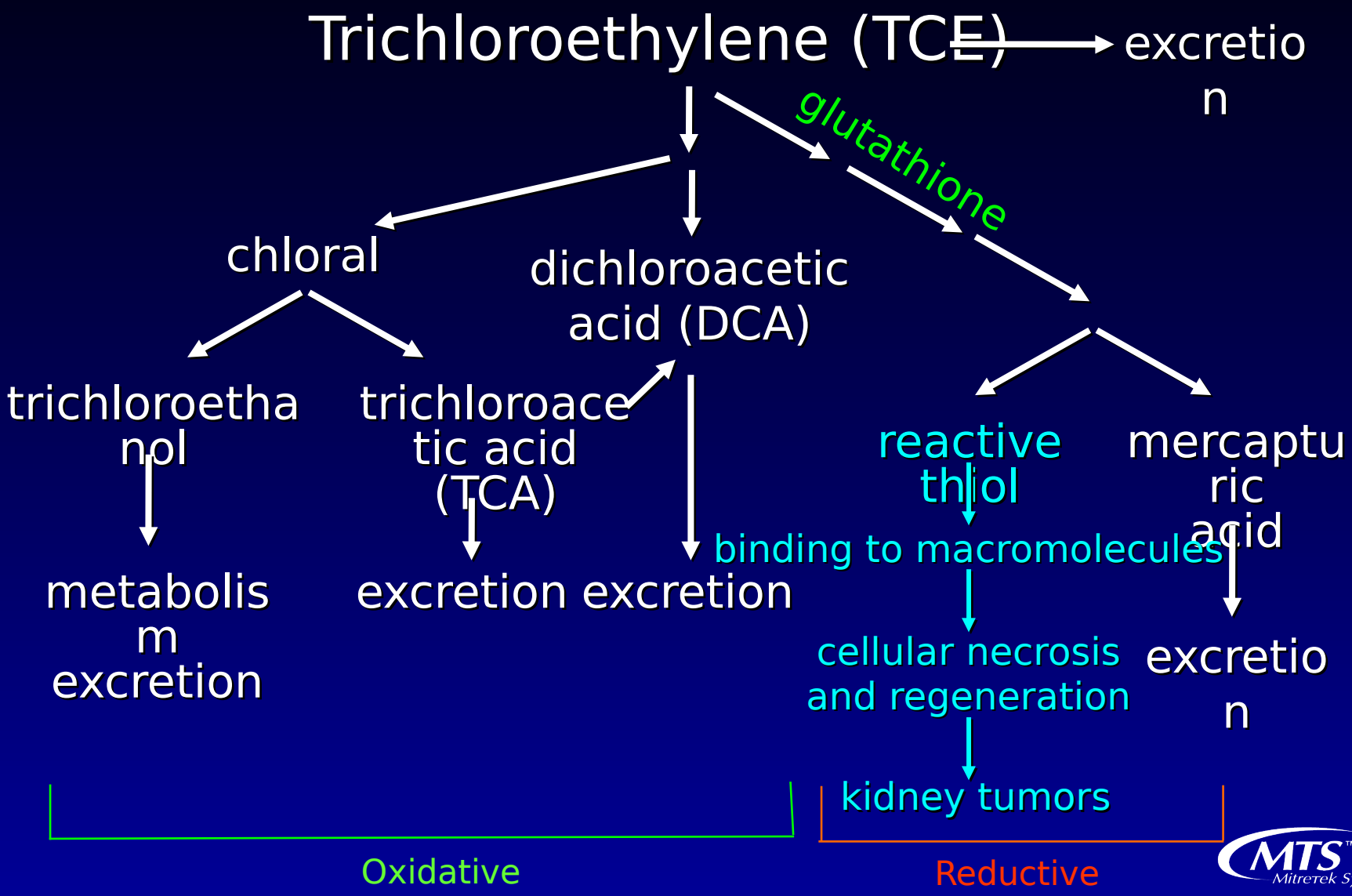
Metabolism of TCE



Epidemiology

- Study of the distribution and determinants of disease
- Identifies factors that
  - Differ between two populations
  - Are sufficiently important to play a determining role in the cause of a disease

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Types of Epidemiology Studies

- Cluster analyses
  - Episodic observations of isolated disease cases, often related to exposure to an agent
- Case-Control Studies
  - Retrospective investigations of histories and habits of persons who developed a disease
- Cohort Studies
  - Longitudinal (prospective or retrospective) investigations of persons exposed to an agent

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Hill Criteria Used to Infer Causality

- Temporal relationship
- Strength of association
- Specificity of association
- Dose-response relationship
- Consistency
- Biological plausibility

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Potential Developmental Toxicity of TCE

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Schema for Developmental Toxicity Risk Assessment

- Evaluate human data
  - Cohort studies
  - Case control studies
  - Other types (e.g., cross sectional, ecologic, prevalence)
- Evaluate mammalian developmental toxicity data
  - Acceptable, robust protocols
  - Reproducibility of findings
  - Compare experimental exposures to expected human exposures
- Evaluate other experimental studies to establish plausibility
  - In vitro assays
  - Non-mammalian studies
  - Mechanism studies
- Apply causality criteria

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Human Data

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Human Data

- No cohort studies
- No case control studies
- Other types (e.g., cross sectional, ecologic, prevalence)
  - Goldberg et al (1990)
    - Ecologic study that made first published claims
    - Reported increased cardiac anomalies among babies born in a region of AZ with TCE-contaminated water (6-239 ppb)
    - Combined 33 malformations (includes 12 vessel malformations)
    - Exposure not quantified (especially during critical period); rather assumed
    - Confounding factors largely uncontrolled

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Epidemiology Studies

Location/ Reference	TCE concentration	Comments and Study Limitations
Tucson Valley, AZ (Goldberg et al., 1990)	6-239 ppb	• Population differences not fully considered. • Rate of CHD in TCE-areas less than historical background.
Northern NJ (Bove et al., 1995)	55 ppb	• At $p \leq 0.05$, no statistically significant increase in CHD attributable to TCE. • Rate of CHD in control group < historical background.
Woburn, MA (Lagakos et al., 1986)	267 ppb	• No statistically significant association
Milwaukee, WI (Yauck et al., 2004)	Not indicated	• Increased risk of CHD to babies from women older than 38 yrs •Small number of cases •Confounding risk factors (age) not

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Mammalian Developmental Toxicity Data

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Mammalian Studies Suggesting a Positive Correlation between TCE and CHD

Reference	TCE Concentration	CHD Effect Level	Animal Model and N	Exposure Route
Dawson et al., 1990	**15 ppm** **1500 ppm**	**1500 ppm§**	**Sprague Dawley rats** **N=10-17/group**	**Intrauterine pump** **(GD 0-21)**
Dawson et al., 1993†	**1.5 ppm** **1100 ppm**	**1100 ppm§**	**Sprague Dawley rats** **N=12-20/group**	**Drinking water** **(GD 0-21)**
Johnson et al., 2003†	**2.5 ppb** **250 ppb** **1.5 ppm** **1100 ppm**	**250 ppb*** **1100 ppm***	**Sprague Dawley rats** **N=10-17/group**	**Drinking water** **(GD 0-21)**

* Statistically significant on a per-litter basis
§ Statistically significant on a per-litter basis

† Some of the data presented in Dawson, 1993 was again presented in Johnson et al., 2003; Also, both studies use the Dawson method of dissection.

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Animal Studies which Do Not Support an Association between TCE and CHD

TCE Inhalation:

Reference	Vapor TCE Conc.	Animal Model/N	Exposure
Schwetz et al., 1975	**300 ppm**	**Sprague Dawley rats** **N=20-35/group**	**Inhalation 7 hrs/day**
Dorfmeuller et al., 1979	**1800 ± 200 ppm**	**Long Evans rats** **N=30/group**	**Inhalation before, during, or before & during pregnancy**
Hardin et al., 1981	**500 ppm**	**Sprague Dawley rats and Wistar rats** **N=20-35/group** **New Zealand rabbits** **N=15-20/group**	**Inhalation 6-7 hrs/day**
Healy et	**100 ppm**	**Wistar rats/group**	**Inhalation 4**

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Animal Studies which Do Not Support an Association between TCE and CHD (concluded)

TCE Oral Exposure:

Reference	TCE Dose	Animal Model/N	Exposure Route
National Toxicology Program, 1985	100, 300, and 700 mg/kg/day TCE†	~20 Swiss CD-1 mice/group	Maternal oral exposure
National Toxicology Program, 1986	76, 156, and 289 mg/kg/day TCE†	~20 Fisher 344 rats/group	Maternal oral exposure
Cosby and Dukelow, 1992	24 and 240 mg/kg/day†	~10 B6D2F1 mice/group	Oral gavage (GD 1-5, 6-10, or 11-15)
Fisher et al., 2001	300 and 500 mg/kg/day TCE	19-20 Sprague Dawley rats per group	Oral gavage (GD 6-15)

† TCE doses exceed those yielding positive results in Dawson, 1989, 1993 and Johnson, 2003

Experimental Studies in Additional Mammalian Species

Species	Agent	(Route) Dose	Stage	Fetuses: CV Malformation (Incidence)	Litters: CV Malformation (% litters)	Reference
Swiss Webster Mouse	TCE	(Inhalation) Control: Filtered air 300 ppm	GD 6-15	0/26=0% 0/12=0%	0 0	Schwetz et al., 1975
New Zealand White Rabbit	DCE	(Inhalation) Control: Filtered air 80 ppm 160 ppm	GD 6-18	0/44=0% 0/156=0% 1/37=2.7%	0 0 8.3	Murray et al., 1979
New Zealan	TCE	(Inhalatio	GD			Hardin

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Considerations of the Developmental Toxicity Data

- Cited human data do not support a causal association between TCE exposure and birth defects
- Data from early animal studies have been used without critical evaluation
- Robust studies in animals fail to confirm earlier findings of heart malformations in rats

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A Way Forward

- Previously generated data are conflicting
  - Well-conducted studies are negative
  - Positive studies have serious flaws
    - Lack of consistency among reported defects
    - Low numbers of specific defects
- Group reported defects to increase robustness
  - Use underlying morphogenetic processes to categorize defects
  - Analyze all of the data to find a potential common denominator
  - Use the results, if positive, to generate hypotheses that can be tested

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Does TCE Cause a Specific Type of CHD?

Reference	Concentration of TCE-Related Substance	Type of Study	Predominant Type of CHD Reported
Tikkanen and Heinonen, 1988	No specific concentrations of compounds indicated; study examines effect of hydrocarbon solvents	Epidemiological; Case-control	• Ventricular Septal Defect (VSD; specific type not indicated)
Smith et al., 1989	300-1,800 mg/kg/day trichloroacetic acid	Animal study: Long Evans rat	• Levocardia • VSD (specific type not indicated)
Smith et al., 1992	14-2,400 mg/kg/day of dichloroacetic acid (DCA)	Animal study: Long Evans rat	• Levocardia • Defects between ascending aorta and right ventricle • VSD (specific type not indicated)
Epstein et	1,900-3,500 mg/kg/day	Animal study:	• VSD type I

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Predominant Developmental Mechanisms during Cardiogenesis

- Cellular migration
- Extracellular matrix formation
- Hemodynamics
- Targeted growth
- Cell death
- Visceral situs

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Does TCE Perturb a Particular Developmental Mechanism?

Reference	Concentration of TCE-Related Substance	Type of Study	Predominant Type of CHD	Developmental Process Likely to be Disturbed
Tikkanen and Heinonen, 1988	None reported; examined effect of hydrocarbon solvents	Epidemiological: Case-control	• Ventricular Septal Defect (VSD; specific type not indicated)	• Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics
Smith et al., 1989	300-1,800 mg/kg/day trichloroacetic acid	Animal study: Long Evans rat	• Levocardia • VSD (specific type not indicated)	• Positional information and cardiac looping • Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics
Smith et al., 1992	14-2,400 mg/kg/day of dichloroacetic acid (DCA)	Animal study: Long Evans rat	• Levocardia • Defects between ascending aorta and right ventricle • VSD (specific type not indicated)	• Positional information and/or cardiac looping • Cell migration and/or extracellular matrix formation • Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics
Epstein et al., 1992	1,900-3,500 mg/kg/day DCA	Animal study: Long Evans rat	VSD type I VSD type II	• Cell migration • Hemodynamics

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Distribution of CHD Types and Perturbed Developmental Process

Type of Congenital Heart Defect	% CHD Patients with this Defect	Developmental Process
Ventricular septal defects	15-30%	Cell migration, cell death, extracellular matrix formation, and/or hemodynamics
Tetralogy of Fallot	9-14%	Cell migration
Transposition of the great arteries	10-11%	Cell migration
Atrioventricular septal defects	4-10%	Extracellular matrix formation
Coarctation of the aorta	8-10%	Hemodynamics
Hypoplastic left heart syndrome	4-8%	Hemodynamics

Information obtained from American Heart Association (2005) and Clark (1986, 1992).

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Summary of Selected Mechanism Studies

Reference	TCE Conc	Experimental model	Findings	Comments
Boyer et al., 2000	50-250 ppm	*In vitro* chick atrioventricular canal model	• Dose-dependent ↓ in epithelial-mesenchymal cell transformation at 50-250 ppm. • ↓ expression of 2 genes involved in cardiac development at 250 ppm.	• Nearly impossible to obtain these physiological levels of TCE through drinking water; by inhalation, need exposure to 36,000-180,000 ppm.
Collier et al., 2003	110-1100 ppm	Tissues of TCE-treated rats born to TCE treated mothers	The authors report ↑ expression of 2 genes assoc. with stress response/homeostasis and ↓ expression of genes associated with extracellular matrix.	•Very few samples had up or down regulated genes (typically 1-2) •No dose response relationship • No statistical analysis was performed.
Ou et al., 2003	0.05-100 µM (6.5-13,000 ppb)	*In vitro*-Endothelial cell culture	• TCE ↓ endothelial cell growth at 0.1-100 µM (13-13000 ppb). • TCE ↓ inhibits the interaction between eNOS and hsp90 at 5 µM (650 ppb.	• Requires drinking only water with 350 ppm TCE to achieve this exposure to endothelial cells.

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Conclusions Concerning TCE and CHD

- Extant data do not suggest increased risk of CHDs
- Correlations claimed between TCE and CHDs
  - flawed studies
  - performed with concentrations of TCE that are not environmentally relevant
- The MCL for TCE protects against CHDs
  - several orders of magnitude below the concentrations that might harm the developing heart.

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Potential Carcinogenicity of TCE

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Decision Tree Method for Potential Carcinogens

- Does TCE damage DNA?
  - Bacteria
  - Mammalian cells
- Is exposure to TCE associated with elevated human cancers?
  - Target organs
  - Dose-related incidences
  - Consistency
- Does TCE cause cancer in animals?
  - Threshold?
  - Similar metabolism in humans?

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DNA Damage ?

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Results of Genotoxicity Studies

- TCE and oxidative metabolites
  - Many in vitro and in vivo studies
  - Negative or weakly positive
- Reductive metabolites

  (via glutathione conjugation)
  - Positive
  - Not quantitatively important in humans

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Available Human Data

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Selected Case-Control Studies of TCE and Kidney Cancer

Cases	Controls	Exposure	Results & Comments	Reference
12 (Mass. transformer plant)	1202 (from same employer)	Work histories, job exposure matrix	Risk of kidney cancer ⟺	Greenland et al. (1994)
438 (Minn.)	687 (population)	Work histories, job exposure matrix	Risk of renal cell carcinoma ⟺ Gender difference? ⟺	Dosemeci et al. (1999)
935 (Germany)	4298 (population)	Job title/tasks, job exposure matrix	Risk of renal cell carcinoma ↑ No evidence of gender difference	Pesch et al. (2000)
58 (Germany)	84 (hospital)	Work histories, interviews	OR 10.8 (3.36-34.75) for renal cell carcinoma High, long-term	Vamvakas et al. (1998)

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Criteria for Inclusion of Cohort Studies in Further Analysis

- Investigation of cancer outcomes
- Cohort size of >750
- Assessment of TCE exposure
- Follow-up period of > 25 years

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Selected Cohort Studies of TCE

Cohort	Size	Years	Exposure	Results & Comments	Reference
Hill AFB, UT	7204	38	Job titles/ descriptions, interviews, historical records	Cancer incidence ↔ Cancer mortality ↔ Kidney cancer incidence ↔ Kidney cancer mortality ↔ ↔	Blair et al. (1998)
Hughes Aircraft plant, AZ	4733	43	Job exposure matrix	Cancer mortality Kidney cancer mortality ↔ Healthy worker effect ↓	Morgan et al. (1998)
Lockheed Martin aircraft plant, CA	2267	36	Job descriptions, interviews, historical records	Cancer mortality ↔ Kidney cancer mortality	Boice et al. (1999)

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Selected Cohort Studies of TCE (Concluded)

Cohort	Size	Years	Exposure	Results & Comments	Reference
Swedish workers	1670	32	Urinary TCA	Cancer mortality ♂ ⟷ Cancer mortality ♀ ⟷ Kidney cancer incidence ⟷	Axelson et al. (1994)
Finnish workers	3089	26	Urinary TCA	Cancer mortality ⟷ Cancer incidence ⟷ Kidney cancer incidence ⟷	Anttila et al. (1995)
Danish workers	803	28	Air TCE, urinary TCA	Cancer incidence ⟷ Kidney cancer incidence	Hansen et al. (2001)
German cardboard factory	169	37	Job histories, interviews	Kidney cancer incidence ↑ 5-10x ⟷ Kidney cancer mortality ↑ High, long-term exposures Methodological flaws	Henschler et al. (1995)

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Some Cautions Regarding the Henschler Study

- Small cohort size (N = 169) limits statistical robustness
- Study emanated from a cluster
  - Formed the basis for the hypothesis
  - Purists would have excluded the cluster from a longitudinal study
- Unexposed cohort exhibited 9-fold increase in brain cancer deaths
  - Attributed to “observer sensitivity bias”

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Some Cautions Regarding the Henschler Study (Continued)

- Critics call attention to the misclassifying of the renal pelvis tumor with renal cell tumors
  - Different location
  - Different tissue of origin
  - Recognized by the authors and deliberately done

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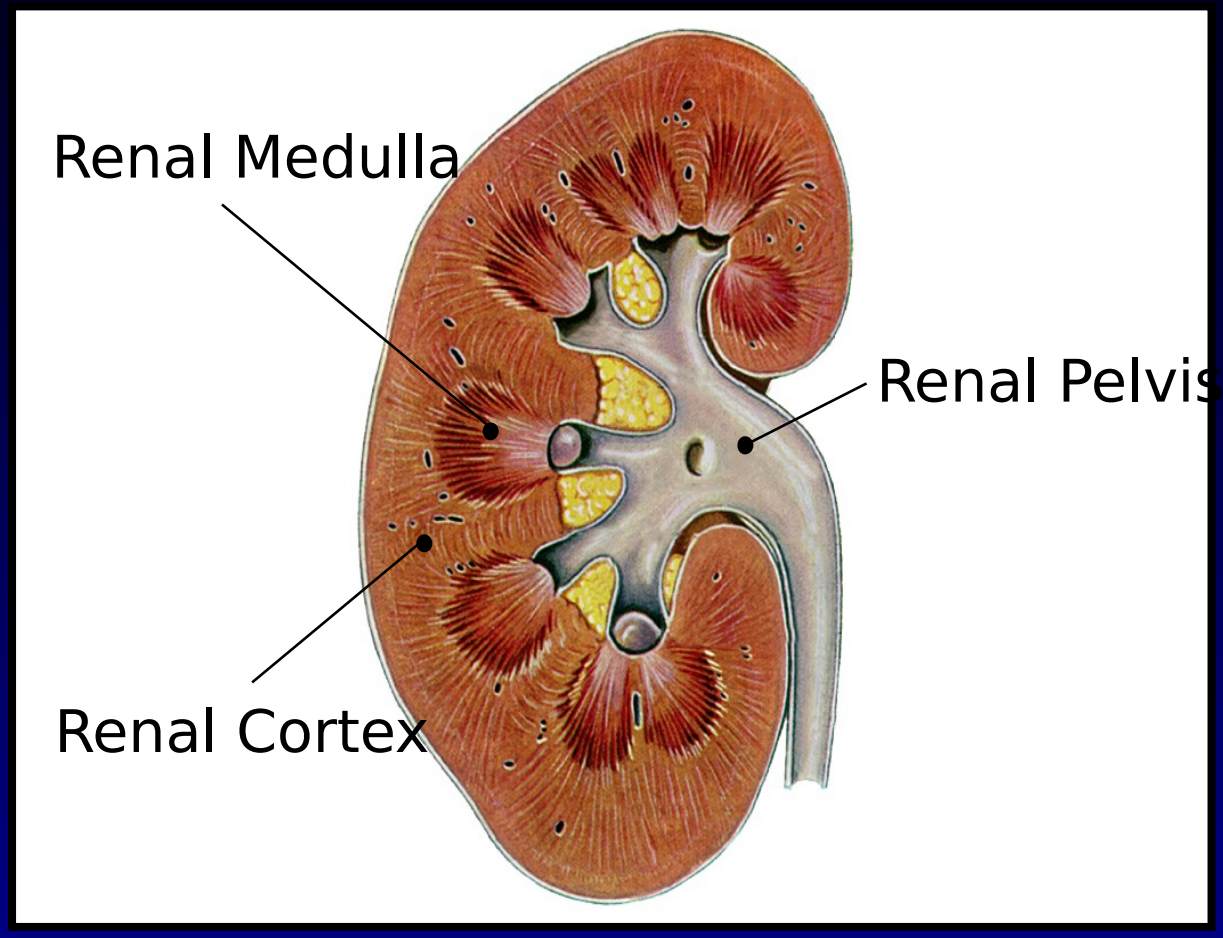
Sagittal Section of the Kidney



Some Cautions Regarding the Henschler Study (Concluded)

- There is a lack of a dose-response
  - Three renal cell tumors in the low-exposure group; latency periods of 18-19 yr
  - One renal cell tumor and one renal pelvis tumor in the high-exposure group; latency periods of 34 yr
- BUT - Two additional renal cell cancers occurred during the period between the end of the study and final publication

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Assessment of Selected Human Studies

- Most data do not support an association between TCE exposure and kidney cancer in humans
  - Among selected case-control studies of TCE
    - 3 negative
    - 1 positive
  - Cohort studies which met inclusion criteria
    - 6 negative
  - One small “cohort” (?) study positive
- Newer studies have shown positive but not statistically robust effects
- Investigations of VHL tumor suppressor gene mutations
  - Positive findings in some studies
  - Small number of cases
  - Problematic mechanism (TCE appears to be nongenotoxic)

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Results from Animal Studies

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Chronic Inhalation Studies in Animals

- TCE at 0, 100, 300, 600 ppm
  7 hr/d, 5 d/wk, for 78 wk
  to Swiss and B6C3F1 mice
  - Male Swiss: dose-related lung and liver tumors
  - Female B6C3F1: dose-related lung tumors
- TCE at 0, 100, 300, 600 ppm
  7 hr/d, 5 d/wk, for 104 wk
  to Sprague-Dawley rats
  - Males: dose-related kidney toxicity and tumors
- Negative results in hamsters

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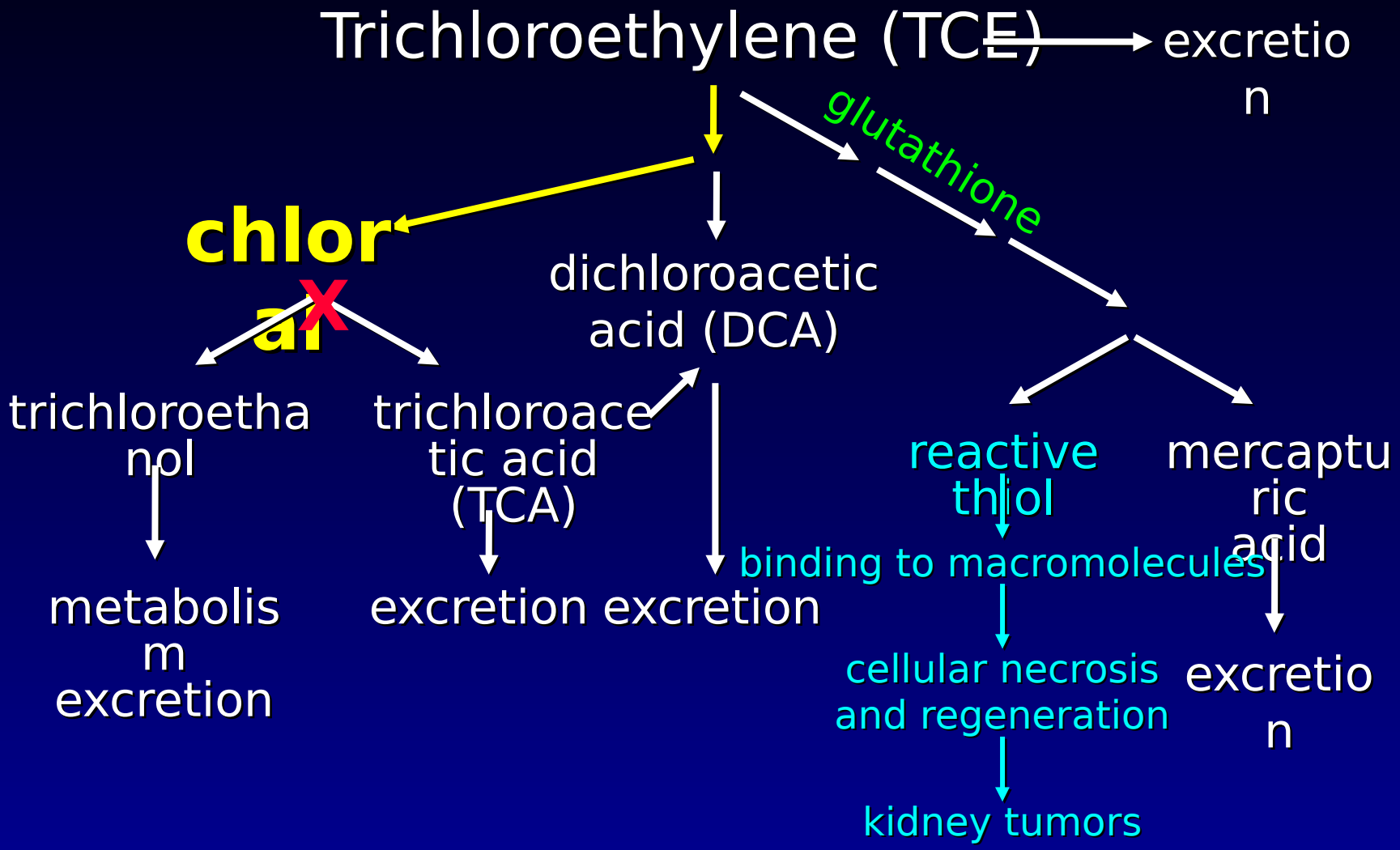
Metabolism of TCE in Mouse Lung



Chronic Oral Studies in Animals

- Early studies confounded by carcinogenic stabilizers
- TCE at 0 or 1000 mg/kg
  5 d/wk for 103 wk
  to B6C3F1 mice
  - Both sexes: increased liver tumors and kidney toxicity
- TCE at 0, 500, or 1000 mg/kg
  5 d/wk for 103 wk
  to 5 strains of rats
  - Inadequate for assessing carcinogenicity
  - Both sexes of all 5 strains: increased kidney toxicity
  - Males of 2 strains: increased kidney tumors

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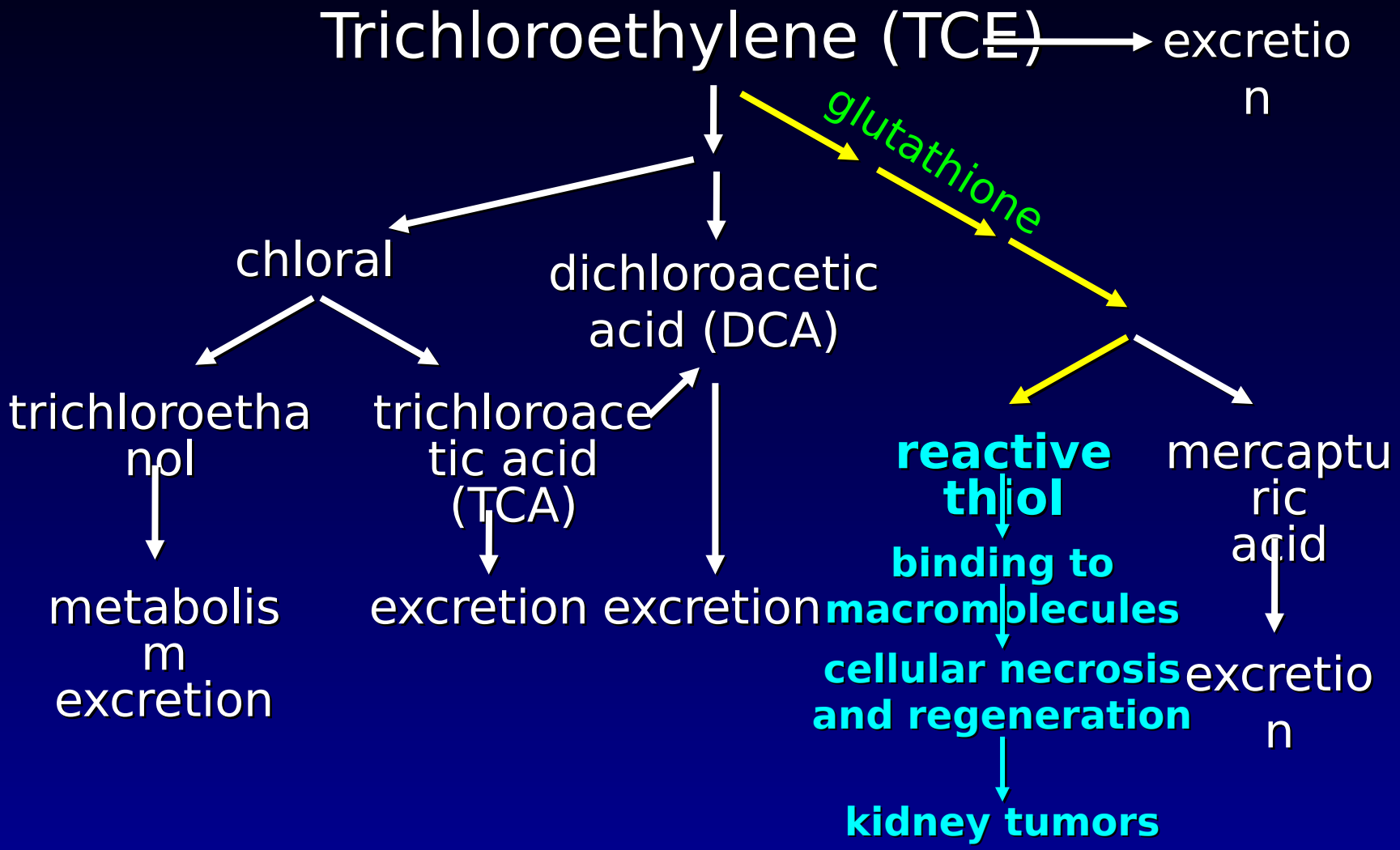
Metabolism of High Dose TCE in Rat Kidney



Likely Mechanism for Renal Tumor Development in Rats

Chronic High Dose

Trichloroethylene → Kidney Necrosis → Regenerative Hyperplasia → **Tumors**

Chronic Low Dose

Trichloroethylene → No Obvious Changes in Tissues → **Normal Tissues (No Tumors)**

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Bases for a Conservative Stance

- Rely heavily on the Henschler study because it alleges cancer outcome in humans
- Emerging concept of “opportunistic carcinogen” is being applied to TCE
- New molecular findings (VHL mutations) in humans could be consistent with a possible cancer effect
  - Unvalidated
  - Small numbers of subjects
- Speculations about non-cancer endpoints in animals (PPAR-α) suggest the absence of a toxicity threshold
  - Mechanism unlikely in humans
- Conservative (over-protective) position is easy to defend

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Probable Consequences of Conservative Assessment

- Maximum contaminant level (MCL) for drinking water will decrease
  - Likely range will be 0.3 – 1.0 µg/L (0.3 – 1.0 ppb)
- Closed sites may be re-examined and, potentially, re-opened
- Soil vapor intrusion into crawl spaces, basements, and buildings will result in new clean-up challenges

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Thoughts on Human Condition

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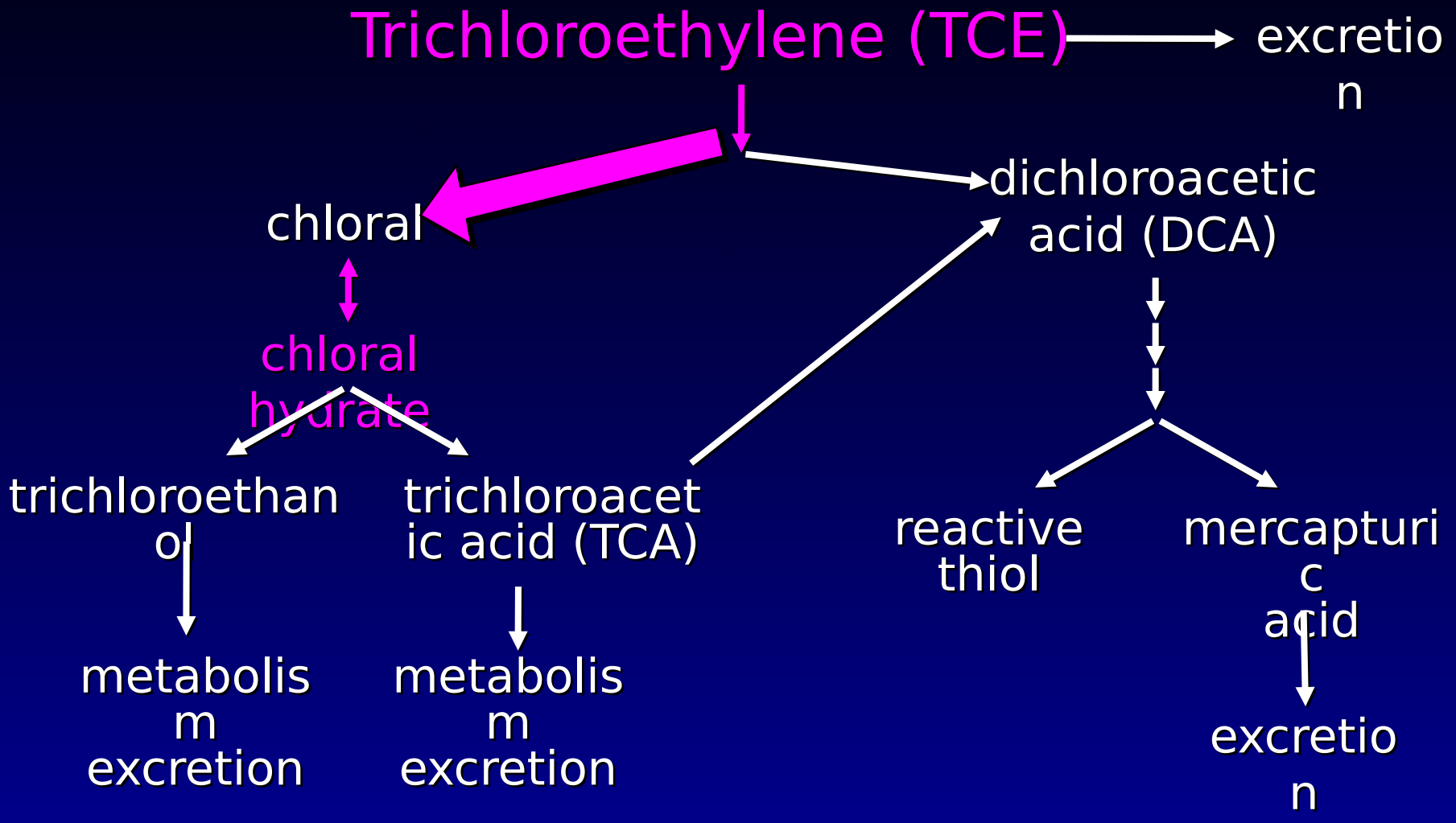
Metabolism of TCE in Humans



A Perspective on Amounts

- Pediatric dose of chloral hydrate = 900 mg
- Equivalent amount of TCE = 715 mg
- How much water is required to dilute this to the current MCL of TCE (5 μg/L)?
  - ~37,730 gallons
  - A swimming pool 35’ long x 18’ wide x 8’ deep
- At 2 L/day, how long for a single person to drink it all?
  - Over 195 years
  - (At 1 μg/L, it would take nearly a millennium!!)

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